

Set	Items	Description
S1	89	S (ANTIBOD? (4N) CAPSID?) (S) (RECOMBINANT (4N) VIRUS?)
S2	41	S (ANTIBOD? (4N) CAPSID?) (6N) (RECOMBINANT (4N) VIRUS?)
S3	29	RD (unique items)
S4	26	S S3 NOT PY>2002
S5	498	S E1:E8,E17:E23
S6	54	S S5 AND CAPSID
S7	18	RD (unique items)
S8	4	S S7 NOT PY>2002
S9	1	S S5 AND CAPSID? AND ANTIBOD?

4/3,AB/2 (Item 2 from file: 155) [Links](#)

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MEDLINE(R)

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13856984 PMID: 11145271

Characterization of monoclonal antibodies generated against Norwalk virus GII capsid protein expressed in Escherichia coli.

Yoda T; Terano Y; Suzuki Y; Yamazaki K; Oishi I; Utagawa E; Shimada A; Matsuura S; Nakajima M; Shibata T

Division of Food Microbiology, Osaka Prefectural Institute of Public Health, Japan.

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Microbiology and immunology (Japan) 2000 , 44 (11) p905-14 , ISSN: 0385-5600--Print

Journal Code: 7703966

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

The Norwalk virus (NV) causes outbreaks of acute non-bacterial gastroenteritis in humans. The virus capsid is composed of a single 60 kDa protein. The capsid protein of NV36 (genogroup II, Mexico virus type) was expressed in an Escherichia coli system and ten monoclonal antibodies (MAbs) were generated against it. The reactivity of these MAbs was characterized using enzyme-linked immunosorbent assay (ELISA) and Western blot (WB) analysis towards 20 overlapping fragments of the NV36 capsid protein expressed in E. coli. All of the MAbs recognized sequential (continuous) epitopes on the three antigenic regions. Six of the 10 MAbs recognized fragment 2 (equivalent residues 31-70), three MAbs recognized fragment 13 (residues 361-403) and one MAb recognized fragment 7 (residues 181-220), suggesting that the N-terminal domain (residues 1-220) may contain more antigenic epitopes than the C-terminal domain (residues 210-548). Furthermore, two MAbs (1B4 and 1F6) reacted in WB with three purified NV strains (genogroup II) derived from patients' stool samples. It was also found that genogroup I recombinant NV96-908 (genogroup I, KY89 type) could be detected as sensitively as recombinant NV36 (genogroup II) by ELISA with a set of the MAbs produced here.

4/3,AB/3 (Item 3 from file: 155) [Links](#)

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13756033 PMID: 11021399

Expression of recombinant capsid proteins of chitta virus, a genogroup II Norwalk virus, and development of an ELISA to detect the viral antigen.

Kobayashi S; Sakae K; Suzuki Y; Ishiko H; Kamata K; Suzuki K; Natori K; Miyamura T; Takeda N
Development Department, Mitsubishi Kagaku Bio-Clinical Laboratories Inc., Tokyo, Japan.

shinkoba@he.mirai.ne.jp

Microbiology and immunology (JAPAN) 2000 , 44 (8) p687-93 , ISSN: 0385-5600--Print

Journal Code: 7703966

Publishing Model Print

Document type: Journal Article; Research Support, Non-U.S. Gov't

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

The second open reading frame (ORF2) gene of the Chitta virus (CHV) was cloned to construct a recombinant baculovirus. The CHV ORF2 is predicted to encode a capsid protein of 535 amino acids (aa). CHV showed a high aa identity in the capsid region with genogroup II Norwalk virus (NV) (65-85%), but a low aa identity with genogroup I NV (44-46%). Phylogenetic analysis of the ORF2 gene demonstrated that CHV is genetically closely related to the Hawaii virus included in genogroup II NV. The recombinant capsid protein of CHV (rCHV) self-assembled to form empty virus-like particles (VLPs) when expressed in insect cells with the recombinant baculovirus. An enzyme-linked immunosorbent assay (ELISA) based on antisera to rCHV was developed to detect CHV antigen in stools. The antigen ELISA appeared to be highly specific to both rCHV and CHV-like strains. In addition, combined use of antigen ELISAs using antibodies against two antigenically distinct recombinant VLPs, the recombinant Chiba virus (rCV) and recombinant Seto virus (rSEV), enabled us to determine the genetic as well as antigenic relationship among these three viruses.

4/3,AB/5 (Item 5 from file: 155) [Links](#)

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MEDLINE(R)

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12483535 PMID: 9434729

Replicon-helper systems from attenuated Venezuelan equine encephalitis virus: expression of heterologous genes in vitro and immunization against heterologous pathogens in vivo.

Pushko P; Parker M; Ludwig G V; Davis N L; Johnston R E; Smith J F

Virology Division, U.S. Army Medical Research Institute for Infectious Diseases, Fort Detrick, Frederick, Maryland 21702, USA.

Virology (UNITED STATES) Dec 22 1997 , 239 (2) p389-401 , ISSN: 0042-6822--Print

Journal Code: 0110674

Publishing Model Print

Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

A replicon vaccine vector system was developed from an attenuated strain of Venezuelan equine encephalitis virus (VEE). The replicon RNA consists of the cis-acting 5' and 3' ends of the VEE genome, the complete nonstructural protein gene region, and the subgenomic 26S promoter. The genes encoding the VEE structural proteins were replaced with the influenza virus hemagglutinin (HA) or the Lassa virus nucleocapsid (N) gene, and upon transfection into eukaryotic cells by electroporation, these replicon RNAs directed the efficient, high-level synthesis of the HA or N proteins. For packaging of replicon RNAs into VEE replicon particles (VRP), the VEE capsid and glycoproteins were supplied in trans by expression from helper RNA(s) coelectroporated with the replicon. A number of different helper constructs, expressing the VEE structural proteins from a single or two separate helper RNAs, were derived from attenuated VEE strains. Regeneration of infectious virus was not detected when replicons were packaged using a bipartite helper system encoding the VEE capsid protein and glycoproteins on two separate RNAs. Subcutaneous immunization of BALB/c mice with VRP expressing the influenza HA or Lassa virus N gene (HA-VRP or N-VRP, respectively) induced antibody responses to the expressed protein. After two inoculations of HA-VRP, complete protection against intranasal challenge with influenza was observed. Furthermore, sequential immunization of mice with two inoculations of N-VRP prior to two inoculations of HA-VRP induced an immune response to both HA and N equivalent to immunization with either VRP construct alone. Protection against influenza challenge was unaffected by previous N-VRP immunization. Therefore, the VEE replicon system was characterized by high-level expression of heterologous genes in cultured cells, little or no regeneration of plaque-forming virus particles, the capability for sequential immunization to multiple pathogens in the same host, and induction of protective immunity against a mucosal pathogen.

4/3,AB/7 (Item 7 from file: 155) [Links](#)

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12038367 PMID: 8970972

Immunogenicity of an aphthovirus chimera of the glycoprotein of vesicular stomatitis virus.

Grigera P R; Garcia-Briones M; Periolo O; la Torre J L; Wagner R R

Centro de Virologia Animal (CEVAN-CONICET), Buenos Aires, Argentina.

Journal of virology (UNITED STATES) Dec 1996 , 70 (12) p8492-501 , ISSN: 0022-538X--

Print Journal Code: 0113724

Contract/Grant No.: R37 AI 11112; AI; United States NIAID

Publishing Model Print

Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

An oligodeoxynucleotide coding for amino acids 139 through 149 of antigenic site A (ASA) of the VP1 capsid protein of the foot-and-mouth disease virus C3 serotype (FMDV C3) was inserted into three different in-frame sites of the vesicular stomatitis virus New Jersey serotype (VSV-NJ) glycoprotein (G) gene cDNA present in plasmid pKG97 under control of the bacteriophage T7 polymerase promoter. Transfection of these plasmids into CV1 cells coinfecting with the T7 polymerase-expressing vaccinia virus recombinant vTF1-6,2 resulted in expression of chimeric proteins efficiently reactive with both anti-FMDV and anti-VSV G antibodies. However, in vitro translation of transcripts of these VSV-G/FMDV-ASA chimeric plasmids resulted in proteins that were recognized by anti-G serum but not by anti-FMDV serum, indicating a requirement for in vivo conformation to expose the ASA antigenic determinant. Insertion of DNA coding for a dimer of the ASA undecapeptide between the VSV-NJ G gene region coding for amino acids 160 and 161 gave rise to a chimeric ASA-dimer protein designated GF2d, which reacted twice as strongly with anti-FMDV antibody as did chimeric proteins in which the ASA monomer was inserted in the same position or two other G-gene positions. For even greater expression of chimeric VSV-G/FMDV-ASA proteins, plasmid pGF2d and a deletion mutant p(delta)GF2d (G protein deleted of 324 C-terminal amino acids) were inserted into baculovirus vectors expressing chimeric proteins GF2d-bac and deltaGF2d-bac produced in Sf9 insect cells. Mice vaccinated with three booster injections of 30 microg each of partially purified GF2d-bac protein responded by enzyme-linked immunosorbent assay with FMDV antibody titers of 1,000 units, and those injected with equivalent amounts of deltaGF2d-bac protein showed serum titers of up to 10,000 units. Particularly impressive were FMDV neutralizing antibody titers in serum of mice vaccinated with deltaGF2d-bac protein, which approached those in the sera of mice vaccinated with three 1-microg doses of native FMDV virions. Despite excellent reactivity with native FMDV, the anti-deltaGF2d-bac antibody present in vaccinated mouse serum showed no capacity to bind to sodium dodecyl sulfate (SDS)-denatured FMDV virions and only minimal reactivity with VP1 protein by Western blotting (immunoblotting) after SDS-polyacrylamide gel electrophoresis. It was also shown in a competitive binding assay that a synthetic ASA undecapeptide, up to concentrations of 200 microg/ml, was quite limited in its ability to inhibit binding of anti-deltaGF2d-bac antibody to native FMDV virions. These results suggest that the chimeric VSV-G/FMDV-ASA proteins mimic the capacity of FMDV to raise and react with neutralizing antibodies to a restricted number of ASA conformations present on the

surface of native FMDV particles.

4/3,AB/9 (Item 9 from file: 155) [Links](#)

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11703327 PMID: 8599210

Antigenic mapping of the recombinant Norwalk virus capsid protein using monoclonal antibodies.

Hardy M E; Tanaka T N; Kitamoto N; White L J; Ball J M; Jiang X; Estes M K

Baylor College of Medicine, Houston, Texas 77030, USA.

Virology (UNITED STATES) Mar 1 1996 , 217 (1) p252-61 , ISSN: 0042-6822--Print Journal Code: 0110674

Contract/Grant No.: AI-30448; AI; United States NIAID; AI-38036; AI; United States NIAID; T32 DK-07664; DK; United States NIDDK

Publishing Model Print

Document type: Journal Article; Research Support, U.S. Gov't, P.H.S.

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Norwalk virus (NV) is the prototype strain of a group of noncultivable caliciviruses that infect humans and cause outbreaks of epidemic acute nonbacterial gastroenteritis. The NV virion is composed of 180 copies of a single structural protein that, when expressed in insect cells infected with a recombinant baculovirus, assembles into empty recombinant Norwalk virus-like particles (rNV VLPs) which are morphologically and antigenically similar to native NV. We have begun to dissect the antigenic structure of the rNV particles using monoclonal antibodies made to the rNV VLPs. Ten MAbs made to rNV particles were characterized for their reactivity as detector antibodies by ELISA, as capture antibodies in an ELISA to detect NV in stools, by Western blot, and by immunoprecipitation. Seven of the MAbs recognize discontinuous epitopes, requiring the rNV capsid protein to remain at least partially folded, while the other three recognize continuous epitopes. Eight of the MAbs map to the C-terminal half of the capsid protein as they react by Western blot and by immunoprecipitation with a 32K trypsin cleavage product of the full-length 58K capsid protein, suggesting that the C-terminal half of the capsid protein may contain the immunodominant epitopes. The three MAbs that recognize continuous epitopes map to the extreme C terminus of the capsid protein, between amino acids 457 and 530, in a region that is relatively conserved among different human calicivirus capsid proteins. These MAbs which were assigned into three antigenic groups will be useful as tools to further dissect the structural and antigenic topography of the NV virion, and as unlimited reagents to detect NV in diagnostic assays.

4/3,AB/11 (Item 11 from file: 155) [Links](#)

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11273867 PMID: 7530391

Immunization of mice with HPV vaccinia virus recombinants generates serum IgG, IgM, and mucosal IgA antibodies.

Hagensee M E; Carter J J; Wipf G C; Galloway D A

Fred Hutchinson Cancer Research Center, Seattle, Washington 98104-2029.

Virology (UNITED STATES) Jan 10 1995 , 206 (1) p174-82 , ISSN: 0042-6822--Print

Journal Code: 0110674

Contract/Grant No.: AI29363; AI; United States NIAID; CA42792; CA; United States NCI
Publishing Model Print

Document type: Journal Article; Research Support, U.S. Gov't, P.H.S.

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

To assess the utility of vaccinia virus recombinants in the development of an immune response against HPV capsid antigens, 5-week-old C57B16 female mice were administered either purified HPV 1 capsids produced by a vaccinia virus recombinant or the recombinant vaccinia virus itself. Animals were boosted at Week 4 with either agent. Mice developed a serum IgG antibody response in all the administration protocols that was directed mainly against native L1 epitopes. Mice injected initially with the vaccinia virus recombinant and boosted with purified capsids had a higher titer antibody response ($P = 0.024$) with more mice responding to a greater extent. All mice produced a serum IgM response that preceded the IgG response by approximately 2 weeks and lasted 1-3 weeks. The IgM response was directed against native L1 epitopes. Although no serum IgA was detected, IgA could be detected in vaginal secretions of mice that were immunized or boosted with the vaccinia virus vector. These results indicate that an extensive humoral immune response to HPV can be elicited using vaccinia virus recombinants.

4/3,AB/12 (Item 12 from file: 155) [Links](#)

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10910326 PMID: 8151302

Expression of functional protease and subviral particles by vaccinia virus containing equine infectious anaemia virus gag and 5' pol genes.

McGuire T C; O'Rourke K I; Baszler T V; Leib S R; Brassfield A L; Davis W C

Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University.

Journal of general virology (ENGLAND) Apr 1994 , 75 (Pt 4) p895-900 , ISSN: 0022-1317--

Print Journal Code: 0077340

Contract/Grant No.: AI24291; AI; United States NIAID

Publishing Model Print

Document type: Journal Article; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, P.H.S.

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Cells infected with vaccinia viruses expressing the equine infectious anaemia virus (EIAV) gag gene (VGag) or gag plus the 5' pol encoding protease (VGag/PR) were evaluated with monoclonal antibody to a p26 capsid protein linear epitope (QEISKFLTD). Both recombinant viruses expressed Gag precursor protein (55K) whereas only VGag/PR expressed a detectable Gag-Pol fusion protein (82K) with a functional protease, shown by subviral particles containing processed p26. Horses inoculated with VGag/PR produced antibodies reactive with EIAV Gag proteins.

4/3,AB/13 (Item 13 from file: 155) [Links](#)

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10889023 PMID: 7510082

Use of HPV 1 capsids produced by recombinant vaccinia viruses in an ELISA to detect serum antibodies in people with foot warts.

Carter J J; Hagensee M B; Lee S K; McKnight B; Koutsky L A; Galloway D A

Fred Hutchinson Cancer Research Center, Seattle, Washington 98104-2029.

Virology (UNITED STATES) Mar 1994 , 199 (2) p284-91 , ISSN: 0042-6822--Print Journal Code: 0110674

Contract/Grant No.: AI 29363; AI; United States NIAID; CA42792; CA; United States NCI Publishing Model Print

Document type: Journal Article; Research Support, U.S. Gov't, P.H.S.

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

A sandwich ELISA was developed to detect HPV antibodies using HPV 1 capsids that were purified from recombinant vaccinia virus-infected cells and a monoclonal antibody to the HPV 1 L1 protein. Sera from 91 college-aged women who had been previously screened for HPV 1 antibodies by immune precipitation of capsid proteins were tested by ELISA. A cutoff point was established independently of other criteria based on the assumption that the ELISA values came from a mixture of two Normal distributions representing seropositive and seronegative individuals. It was found that the data fit this model best when the natural log of the ELISA (+0.5 to make all of the values positive) was used. Positive sera were shown to react with a conformational epitope(s) on the L1 protein. In the population reporting foot warts, 16 of 18 (89%) had ELISA values above the cutoff. This compared to 38 of 73 (53%) positives in the population reporting no history of foot warts. The odds ratio for the association of the ELISA reactivity with foot warts was 7.23 (95% CI 1.53, 69.4; $P < 0.01$). There was no significant association between the ELISA reactivity and wart infections reported at other sites. The average of the log ELISA values for individuals never reporting foot warts was -0.223 (SD 0.468), whereas the average value for individuals reporting foot warts within 10 years was 0.191 (SD 0.450) ($P = 0.001$). There was a negative correlation between the magnitude of ELISA reactivity and the time elapsed since the last appearance of foot warts. This apparent loss of seroreactivity over time may indicate that HPV 1 is usually eliminated from the host after infection or that inadequate levels of HPV 1 capsid antigen are produced during latent foot warts to maintain antibody levels.

4/3,AB/18 (Item 2 from file: 73) [Links](#)

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0078872785 EMBASE No: 2002036428

Modified indirect Porcine Circovirus (PCV) type 2-based and recombinant capsid protein (ORF2)-based enzyme-linked immunosorbent assays for detection of antibodies to PCV

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Clinical and Diagnostic Laboratory Immunology (Clin. Diagn. Lab. Immunol.) (United States)
February 5, 2002 , 9/1 (33-40)

CODEN: CDIME ISSN: 1071412X

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Document Type: Journal ; Article Record Type: Abstract

Language: English Summary language: English

Number of References: 49

Postweaning multisystemic wasting syndrome of swine associated with porcine circovirus (PCV) is a recently reported and economically important disease. Simple and reliable diagnostic methods are needed for detecting antibodies to PCV type 2 (PCV2) for monitoring of PCV infection. Here, we report the development of two modified indirect enzyme-linked immunosorbent assays (ELISAs): a PCV2 ELISA based on cell-culture-propagated PCV2 and an ORF2 ELISA based on recombinant major capsid protein. PCV2 and ORF2 ELISA detected antibodies to PCV2 and the capsid protein, respectively, in sera from pigs experimentally infected with PCV2 as early as 14 and 21 days postinoculation (dpi). The kinetics of the antibody response to PCV2 and the major capsid protein were similar. Repeatability tests revealed that the coefficients of variation of positive sera within and between runs for both assays were less than 30%. To validate the assays, PCV2 and ORF2 ELISAs were performed with 783 serum samples of young and adult pigs collected from different herds in the Midwestern United States and compared with an indirect immunofluorescent assay (IIF). Six out of 60 samples collected from nursery and growing pigs in 1987 were positive by both ELISA and IIF. Compared with IIF, the diagnostic sensitivity, specificity, and accuracy of PCV2 and ORF2 ELISAs were similar (>90%). The tests showed no cross-reactivity with antibodies to porcine parvovirus and porcine reproductive and respiratory syndrome virus. There was good agreement between the two ELISAs and between the ELISAs and IIF. The availability of the two ELISAs should accelerate our understanding of the host immune response to PCV2 and facilitate the development of prevention and control strategies by elucidating the ecology of PCV2 within swine populations.

4/3,AB/23 (Item 1 from file: 5) [Links](#)

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10667700 Biosis No.: 199191050591

**BOVINE SEROLOGICAL RESPONSE TO A RECOMBINANT BPV-1 MAJOR CAPSID
PROTEIN VACCINE**

Author: JIN X W (Reprint); COWSERT L; MARSHALL D; REED D; PILACINSKI W; LIM L Y;
JENSON A B

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Journal: Intervirology 31 (6): p 345-354 1990

ISSN: 0300-5526

Document Type: Article

Record Type: Abstract

Language: ENGLISH

Abstract: Four of five groups of Holstein by Angus calves (5 calves/group) were immunized with different formulations of a recombinant BPV-1 DNA vaccine using a BPV-1 major capsid:B-galactosidase fusion protein as the immunogen. Group 5 was not vaccinated. Vaccinated calves received the vaccine on days 0 and 21 of the trial, and calves from all five groups were challenged intradermally with 10¹⁰ BPV-1 particles at each of two different sites on day 56. All calves were bled on days 3, 24, 55, 77, and 104 of the trial, and the sera were tested for reactivity with intact and disrupted BPV-1 particles by ELISA. At the time of challenge with BPV-1 virions (day 56), 19 of 20 vaccinated calves were seropositive for disrupted BPV-1 particles; sera from 3 of 20 calves reacted with intact BPV-1 virions. By day 77, 11 of 19 vaccinated calves had developed antibody titers of intact BPV-1 virions; only 1 calf in group 5 developed antibodies (transiently) against BPV-1 capsid epitopes. After challenge, 24 of 25 calves from the five groups developed intradermal fibromas, the biological end point of this study. Fibromas appeared to increase in size in group 5 (unvaccinated, inoculated controls), whereas most tumors from the four vaccinated groups (1-4) stabilized or decreased in size. Although the calves developed fibromas, 90% of calves (in groups 1-4) developed antibodies against disrupted BPV-1 capsid proteins whereas 58% developed antibodies that reacted with intact virions. The immunologic response of vaccinated calves to intact and disrupted BPV-1 particles appeared to be determined in large part by the various formulations of the vaccine, particularly the adjuvant.

4/3,AB/25 (Item 2 from file: 34) [Links](#)

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04679952 Genuine Article#: UA397 Number of References: 73

RELEASE OF VIRUS-LIKE PARTICLES FROM CELLS INFECTED WITH POLIOVIRUS
REPLICONS WHICH EXPRESS HUMAN-IMMUNODEFICIENCY-VIRUS TYPE-1 GAG
(Abstract Available)

Author: PORTER DC; MELSEN LR; COMPANS RW; MORROW CD

Corporate Source: UNIV ALABAMA,DEPT MICROBIOL/BIRMINGHAM//AL/35294; UNIV
ALABAMA,DEPT MICROBIOL/BIRMINGHAM//AL/35294; EMORY UNIV,SCH MED,DEPT
MICROBIOL & IMMUNOL/ATLANTA//GA/30322

Journal: JOURNAL OF VIROLOGY , 1996 , V 70 , N4 (APR) , P 2643-2649

ISSN: 0022-538X

Language: ENGLISH Document Type: ARTICLE

Abstract: The effectiveness of attenuated poliovirus vaccines when given orally to induce both systemic and mucosal immune responses against poliovirus has resulted in an effort to develop poliovirus-based vectors to express foreign proteins. We have previously described the construction of poliovirus genomes (referred to as replicons) in which the complete human immunodeficiency virus type 1 (HIV-1) gag gene was substituted for the capsid gene (P1) (D. C. Porter, D. C. Ansardi, and C. D. Morrow, J. Virol. 69:1548-1555, 1995). Infection of cells with encapsidated replicons resulted in the expression of a 55-kDa protein. To further characterize the biological features of the HIV-1 Gag proteins expressed in cells infected with encapsidated replicons, we utilized biochemical analysis and electron microscopy. Expression of the 55-kDa protein in cells infected with encapsidated replicons resulted in myristylation of the pr55(gag) protein. The Gag precursor protein was released from infected cells; analysis on sucrose density gradients revealed that the precursor sedimented at a density consistent with that of an HIV-1 virus-like particle. Analysis of replicon-infected cells by electron microscopy demonstrated the presence of condensed structures at the plasma membrane and the release of virus-like particles. These studies demonstrate that poliovirus-based vectors can be used to express foreign proteins which require posttranslational modifications, such as myristylation, and assemble into higher-order structures, providing a foundation for the future use of poliovirus replicons as vaccine vectors.